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SHORT COMMUNICATION

Colonisation of the gut by bifidobacteria is much more common in vaginal deliveries than Caesarean sections

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Keywords

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Micro-organisms start to colonise the infant gut during the first days of life and play an important role in human health throughout life (1). More than 10^{12} bacteria per gram of intestinal content present a barrier against colonisation by pathogens and alien microbes. They are involved in metabolism by degrading nondigestible food remnants, producing vitamins B and K and participating in short-chain fatty-acid metabolism. These bacteria also play a role in the stimulation and development of the immune system. Therefore, the colonisation of a newborn infant's gut is vital and it has a significant influence on the final composition of the resident microbiota in adults.

Beneficial bacteria in the intestinal tract, such as bifidobacteria and lactobacilli, contribute to improved health for months, years or even a lifetime (2,3). The colonisation of the human gut is a complicated process that is dependent on a number of factors, which include the duration of pregnancy, the mother's health, gestational age, antibiotic treatment, hospital hygiene, duration and mode of delivery and type of feeding (4,5). The most significant change in the composition of intestinal microbiota occurs in the first few weeks of life. Bifidobacteria are the predominant group of bacteria in the gut of breastfed and vaginally delivered infants (6). The aim of this study was to determine the influence of the mode of delivery on infant gut colonisation by bifidobacteria. In addition, we sought to identify the predominant gut bacteria of infants born by Caesarean section.

The influence of the mode of delivery – Caesarean section versus vaginal delivery – on the microbial composition of faecal samples from infants aged 10–30 days was examined. We analysed 100 faecal samples from healthy infants of both sexes from the Paediatric Department of Motol University Hospital, Prague, Czech Republic. Of these 100 infants, 61 (36 male and 25 female) were born by vaginal delivery and 39 (20 male and 19 female) were born by Caesarean section. All study subjects were full-term infants who were born in the hospital, and all were exclusively breastfed. None of them had been treated with antibiotics or probiotics before sampling. Samples were collected by their mothers, who had also not been treated with antibiotics during pregnancy.

Fresh faecal samples were aseptically transferred to tubes containing Wilkins–Chalgren broth (Oxoid), transported to the laboratory and analysed within two hours. The samples were serially diluted in the Wilkins–Chalgren broth under anaerobic conditions. Media were prepared using the roll-tube technique in an oxygen-free carbon dioxide environment. Faecal bacteria were detected using selective media, according to the method described by Vlková et al. (7).

Appropriate dilutions of the sample were transferred to sterile petri dishes that were immediately filled with media selective for total anaerobes (Wilkins–Chalgren agar, Oxoid), bifidobacteria (TPY agar from Scharlau, Spain, modified by the addition of 100 mg/L of mupirocin and 1 mL/L of acetic acid), lactobacilli (Rogosa agar, Oxoid), gram-negative anaerobes (Wilkins–Chalgren agar, supplemented with G-N Anaerobe Selective Supplement, both Oxoid) and *Escherichia coli* (TBX, Oxoid). Total anaerobes, gram-negative anaerobes and bifidobacteria were incubated in anaerobic jars (Anaerobic Plus System, Oxoid) at 37°C for 48 h. Lactobacilli were cultivated in microaerophilic

conditions at 37°C for 3 days, and *E. coli* was cultivated aerobically at 37°C for 24 h. Fluorescence *in situ* hybridisation kits specific for *Clostridium butyricum* group (Ribo-Technologie, Groningen, the Netherlands) were used for the quantitative detection of clostridia. After hybridisation, the samples were analysed with a Nikon E-800 epifluorescence microscope and Lucia 5.10 software.

The differences among bacterial counts were evaluated by multiple range comparisons with multiple range tests ($p < 0.01$) using STATGRAPHICS Centurion XV.II (Manugistics, Rockville, MD, USA). This enabled us to calculate the differences in the bacterial counts between the experimental groups. A one-sample Kolmogorov–Smirnov test of composite normality was used to confirm the normal distribution of data. The verification of segregation ratios was calculated using chi-squared tests.

Infants were divided into four groups according to their faecal microbiota composition and their mode of delivery (Table 1). These were the following: infants born vaginally with bifidobacteria dominant in the faecal microbiota (vBif+), infants born vaginally without bifidobacteria (vBif−), infants born by Caesarean section with bifidobacteria dominant in the faecal microbiota (cBif+) and infants born by Caesarean section without bifidobacteria (cBif−). Our results showed that in 86.89% of the samples from the vBif+ groups, bifidobacteria were present at 9.69 ± 0.82 log CFU/g, but no clostridia were detected. However, bifidobacteria were not detected in 13.11% of the samples from the vBif− group. The predominant bacteria detected in these vBif− infants were *E. coli* (8.83 ± 1.03 log CFU/g), gram-negative anaerobes (7.50 ± 1.77 log CFU/g) and clostridia (6.94 ± 1.29 log CFU/g).

Furthermore, bifidobacteria were only detected in 41.03% of samples from cBif+ group compared with 86.89% of the samples from the vBif+ group. In the cBif+ group, bifidobacteria were the predominant bacteria and their counts (9.07 ± 1.24 log CFU/g) were nearly the same as those in the vBif+ group. Moreover, in the faecal samples from infants born by Caesarean section, the lactobacilli counts in the cBif+ group (4.91 ± 3.56 log CFU/g) were about one order of magnitude higher than those in the cBif− group (3.90 ± 2.02 log CFU/g), whereas clostridia were not detected in the cBif+ group.

In infants born by Caesarean section, bifidobacteria were not detected in 58.97% of the faecal samples in the cBif− group. In these samples, the relatively dominant bacteria were *E. coli* (9.40 ± 0.75 log CFU/g), clostridia (7.71 ± 2.51 log CFU/g) and gram-negative anaerobes (7.55 ± 1.40 log CFU/g).

Our study confirmed that the mode of infant delivery had a significant impact on the colonisation of the intestinal tract by bifidobacteria. In infants born by Caesarean section, gut colonisation by lactobacilli can begin after 10 days, while colonisation by bifidobacteria may be delayed by 1 month (3). According to Biasucci et al. (8), the mode of infant delivery has a more pronounced effect on the process of bacterial colonisation than the type of feeding the infant receives. Infants born by Caesarean section have lower numbers of bifidobacteria than vaginally born babies (8) and are often colonised with *Clostridium difficile* (9). Colonisation by clostridia at the age of 1 month was associated with wheeze and eczema in older infants (10). Decreases in bifidobacteria in the gut microbiota of infants has been found to correlate with enteric disorders (11). There are several studies that have shown that probiotics with bifidobacteria reduce the incidence and severity of necrotising enterocolitis caused by clostridia (12,13). Pender et al. (9) also showed that infants born by Caesarean section had lower count of faecal bifidobacteria than vaginally delivered infants. Mikami et al. (14) reported

Table 1 Bacterial counts (mean in log CFU/g ± standard deviation) determined in infant faecal samples using cultivation or FISH*

Group	Number of samples	Age (days)	Total anaerobes	*Bifidobacterium*	*Clostridium**	*Lactobacillus*	Gram-negative anaerobes	*E. coli*
vBif+	53	Mode 25 Median 24.5 Range 10–30	10.23 ± 0.54	9.69 ± 0.82	ND	4.00 ± 2.92	7.81 ± 1.72	8.56 ± 1.05[a]
vBif−	8	Mode 23 Median 23.5 Range 10–30	9.88 ± 0.57	ND	6.94 ± 1.29	4.43 ± 2.13	7.50 ± 1.77	8.83 ± 1.03[ab]
cBif+	16	Mode 20 Median 18 Range 10–30	10.18 ± 0.46	9.07 ± 1.24	ND	4.91 ± 3.56	8.23 ± 0.94	9.16 ± 0.82[ab]
cBif−	23	Mode 20 Median 20 Range 10–30	10.18 ± 0.43	ND	7.71 ± 2.51	3.90 ± 2.02	7.55 ± 1.40	9.40 ± 0.75[b]

ND – not detected; *determined by FISH.
All infants were fully breastfed.
Values in columns with different superscripts differ ($p < 0.01$). The differences among bacterial counts were evaluated by the multiple range comparison with multiple range tests.
vBif +/− infants born vaginal delivery with/without bifidobacteria.
cBif +/− infants born Caesarean section with/without bifidobacteria.

that the type of delivery had a significant effect on the development of gut bifidobacteria in neonates.

Our results have confirmed that the mode of infant delivery has a crucial impact on the composition of intestinal microbiota in early infancy. The counts of bifidobacteria significantly differed between infants born by Caesarean section and those born vaginally. If infants born by Caesarean section had bifidobacteria in their gut microbiota, the levels of bacteria were usually equal to those in infants born by vaginal delivery. However, if infants born by Caesarean section did not have bifidobacteria in their gut microbiota, *E. coli* was relatively dominant along with clostridia and gram-negative bacteria. Similarly, infants born vaginally sometimes had a composition of intestinal microbiota that resembled infants born by Caesarean section with very low counts of bifidobacteria. Given these results, it is well established that the type of delivery in our study had a significant effect on the development of gut bifidobacteria in neonates.

Our findings confirm other reports (3,8,9,15) that differences in delivery mode have been linked to differences in the intestinal microbiota of infants. In conclusion, colonisation of the gut by bifidobacteria in vaginally delivered infants is much more common than in infants delivered by Caesarean section. When we looked at exclusively breastfed infants, their guts were significantly and less frequently colonised with bifidobacteria if they were born by Caesarean section than vaginal delivery. Moreover, bifidobacteria counts in infants born by Caesarean section were similar to those in infants born vaginally. Clostridia were frequently found in infants with the absence of bifidobacteria.

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